

Amendments to the Specification:

Please replace the paragraph beginning at page 7, line 8 with the following amended paragraph:

Tumor antigen or tumor antigen-HspC' expression plasmids including pET32a(+)/PSA, pRSET/PSA-HspC', pET32a(+)/AFP and pRSET/AFP-HspC' were used to transform BL21 (DE3) PLYS or BL21 (DE3) RIL (Promega) *E. coli* host strains. Transformed *E. coli* strains were incubated with shaking at 37°C for overnight. Each culture was inoculated to a fresh AP/LB medium and subsequently incubated with shaking at 37°C or 3 hours. 0.1 mM of IPTG was added to the medium to induce the expression of the fusion protein. The culture was centrifuged at 8,000×g at 4°C for 15 minutes to collect the cells. Expression of the pET-tumor antigen or pRSET-tumor antigen-HspC' was examined by comparison of the induced bacterial lysate with the non-induction bacterial lysate, and further confirmed by western blot analysis.

Please replace the paragraph beginning at page 9, line 2 with the following amended paragraph:

E. coli strain BL21(DE3) containing pRSET/Hsp was grown at 37°C in 1 liter of LB broth containing 50 µg/ml Ampicillin. When absorbance A₆₀₀ reached 0.5-0.6, isopropyl-β-thiogalactoside was added at 1 mM to induce Hsp 70 expression, and the growth was continued for 4 h. Bacterial cells were harvested and stored at -20°C. The cell pellet was suspended in 60 ml of lysis buffer (0.01 M NaH₂PO₄, 0.3 M NaCl, pH 8.0) and broken by sonication. The cell lysates were centrifuged at 15,100×g, 4°C for 15 min, and Hsp70 was solubilized in the supernatant.

Please replace the paragraph beginning at page 9, line 19 with the following amended paragraph:

A polymyxin B agarose column (Pierce, Detoxi-Gel™, 20344) was used to remove endotoxin. The column was regenerated with 5 column volumes of 1% sodium deoxycholate and washed with 5 column volumes of pyrogen-free ddH₂O, then equilibrated with 5 column volumes of PBS. The protein was loaded onto the column at 0.4 ml per ml gel volume, then

incubated for 1 h at 37°C. The protein was eluted with PBS, concentrated by centrifugation (Centricon, Millipore) and assayed for endotoxin levels.

Please replace the paragraph beginning at page 9, line 27 with the following amended paragraph:

Cell pellets were suspended in 60 ml of lysis buffer (0.01 M NaH₂PO₄, 0.3 M NaCl, pH 8.0) and broken by sonication. Inclusion bodies were collected by centrifugation at 15,100×g, 4°C for 15 min, and treated with a washing buffer (2 M Urea, 5 mM EDTA, 5 mM DTT, 2M Urea, 2% Triton X-100, 100 mM Tris, pH 7.0) followed by centrifugation. The inclusion bodies were resuspended in 10 ml denature lysis buffer (6 M guanidine, 0.1 M NaH₂PO₄, 0.01 M Tris-HCl, pH 8.0), and mixed for 2 h at room temperature. The guanidine-solubilized protein was purified using Superdex 200. The column was equilibrated with a buffer (50 mM Tris, pH 7.5, 4 M guanidine, 5 mM DTT), and loaded with 4 ml denature protein sample at a 1 ml/min flow rate. The sample was assayed by SDS-PAGE, and collected in a volume of about 50-60 ml.

Please replace the paragraph beginning at page 10, line 11 with the following amended paragraph:

The denatured AFP-Hsp or PSA-Hsp proteins were refolded by rapidly diluting them into a refolding buffer (100 mM Tris, pH 8.0, 0.5 M L-arginine, 10 mM reduced glutathione, 1 mM oxidized glutathione) to a , final protein concentration was of about 0.5 mg/ml. The sample was incubated at room temperature for 2 h, and dialyzed against PBS overnight at 4°C. A small amount of insoluble material was removed by centrifugation (15,100×g for 15 min), and the soluble protein was concentrated by ultrafiltration using a YM-10 membrane (Amicon). The purity of the protein was analyzed by SDS-PAGE, and the total protein concentration was measured by a BCA protein assay (Pierce).

Please replace the paragraph beginning at page 11, line 14 with the following amended paragraph:

Cells ($5-10 \times 10^5$) were stained with 50 ml fluorescent ~~moeity~~moeity-conjugated antibodies in PBS containing 1% BSA and 0.1% azide, which also served as a washing buffer. The following antibodies were used for the surface staining: MHC molecule I-A/I-E (2G9, Pharmingen), CD80 (B7-1), CD86 (B7-2), CD11c (N418, Pharmingen), and DEC205 (NLDC-145, Pharmingen).

Please replace the paragraph beginning at page 12, line 15 with the following amended paragraph:

CT26/PSA tumor cells were stained with green dye PKH-67 (Sigma) according to the manufacturer's described protocol. Briefly, tumor cells were suspended to 1×10^7 /ml and PKH-67 (2 mg/ml) was added dropwise. After incubation at 37°C for 10 min, cells were washed three times with PBS. For induction of apoptosis, tumor cells (2×10^6) were seeded in a T25 tissue culture flask and 24 h later exposed to 100 cGy of irradiation. DThe dendritic cells were cocultured with apoptotic cells at 37°C for 24 h at 1:1 ratio. The cells were harvested, and the dendritic cells were stained with a PE-labeled anti-CD86 antibody. Two-color flow cytometry was performed to determine the percentage of cells that phagocytosed apoptotic cells.

Please replace the paragraph beginning at page 12, line 25 with the following amended paragraph:

Apoptosis was measured with a terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay. The TUNEL assay was performed by using commercial TdT-FragELTM DNA fragmentation detection kit (Cat. No. QIA33, Oncogen). Briefly, tumors ~~was~~ were cut from mice and paraffin sections were made. After hydration and dehydration, the slides were fixed with 4% paraformaldehyde in PBS and permeabilized with 20 $\mu\text{g}/\text{ml}$ proteinase K in 10 mM Tris, pH 8 for 10 minutes at room temperature. Then the endogenous peroxidases were inactivated by 3% H_2O_2 in methanol for 5 minutes at room temperature. After washing, cells were incubated with biotin-labeled dNTP in the presence of terminal deoxynucleotidyl transferase enzyme solution for 1.5 h at 37°C . After incubation, biotinylated nucleotides were detected by using with a streptavidin-horseradish peroxidase conjugate for 25 minutes at room temperature. Diaminobenzidine reacted with the labeled

sample for 15 minutes at room temperature. Finally, tumor cells were counterstained with methyl green for 3 minutes at room temperature.

Please replace the paragraph beginning at page 14, line 24 with the following amended paragraph:

In order to test phagocytosis ability of BM-DC, CT26 cells were labeled with a fluorescent green dye (PKH-67), and BM-DC were then stained with CD86-PE. The heat-induced necrosis (50°C, 30 min) (Rubartelli et al., 1997, Eur. J. Immunol. 27, 1893-1900), and irradiation-induced apoptotic CT26 tumor cells CT26 tumor cells were cocultured with BM-DC. After 16-20 h, the cocultured cells were analyzed by FACSCalibur, allowing the quantification of phagocytosis. Low temperature (i.e., 4°C for 16 hrs inhibited uptake of CT26 by BM-DC. When BM-DC were mixed with labeled-CT26 cells and incubated at 37°C for 16 hrs, thirty percent of the irradiated cells were phagocytosed by BM-DC. These unexpected results demonstrated that immature dendritic cells could be cultured from BM and retain their phagocytosis ability of uptaking dead tumor cells.